Diagnostic efficacy of tests for the detection of iron overload in chronic liver disease

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The value of tests for the detection of body iron overload was investigated in 8 patients with clinically manifest primary hemochromatosis, 12 patients with cirrhosis and iron overload and 20 patients with liver disease and low or normal iron stores. Iron overload was defined as the presence of stainable iron in more than 50% of hepatocytes in a liver biopsy specimen. The percentages of patients with a true-positive (abnormal) or true-negative (normal) result were: serum iron concentration 65%, transferrin saturation 85%, serum ferritin concentration 78%, serum ferritin:serum glutamic oxaloacetic transaminase (SGOT) index 78%, percent iron absorption 58%, percent iron absorption in relation to serum ferritin concentration 80% and percent iron absorption in relation to serum ferritin:SGOT index 93%. The calculated predictive value of a normal test result for the exclusion of iron overload in patients with liver disease, a group with an assumed prevalence of iron overload of 10%, was 98% to 99% for transferrin saturation and serum ferritin concentration used alone and 100% for these measures used together; the predictive value of an abnormal result for the diagnosis of iron overload was less than 50% for all of the above measures used alone or in combination. Hence, in patients with an increased serum ferritin concentration or transferrin saturation, or both, determination of the hepatocellular iron content of a specimen from a percutaneous liver biopsy is required for the diagnosis of iron overload.

On a étudié la valeur des tests servant à découvrir une surcharge en fer de l'organisme chez 8 patients présentant des signes cliniques évidents d'hémochromatose primaire, chez 12 patients atteints d'une cirrhose et d'une surcharge en fer, et chez 20 patients souffrant de maladie hépatique et ayant des réserves de fer basses ou normales. Une surcharge en fer a été définie par la présence de fer colorable dans plus de 50% des hépatocytes d'une biopsie du foie. Les pourcentages des patients présentant un résultat vraiment positif (valeur anormale) ou un résultat vraiment négatif (valeur normale) ont été les suivants: concentration de fer sérique 65%, saturation à la transferrine 85%, concentration sérique en ferritine 78%, index ferritine sérique:transaminase glutamique oxaloacétique sérique (SGOT) 78%, pourcentage d'absorption du fer 58%, pourcentage d'absorption du fer en rapport avec la concentration sérique en ferritine 80% et pourcentage d'absorption du fer en rapport avec l'index ferritine sérique:SGOT 93%. La valeur prévisionnelle calculée pour un résultat normal d'épreuve visant à exclure une surcharge en fer chez les patients souffrant de maladies hépatiques, chez qui on suppose une prévalence de surcharge en fer de 10%, a été de 98% à 99% pour la saturation à la transferrine et pour la concentration sérique en ferritine lorsque ces mesures ont été utilisés seuls, et de 100% pour les deux mesures utilisées ensemble; la valeur prévisionnelle d'un résultat anormal pour le diagnostic de surcharge en fer a été de moins de 50% pour l'ensemble des mesures précédentes utilisées seules ou en association. Donc, chez les patients présentant une augmentation de la concentration sérique en ferritine ou de la saturation à la transferrine, ou les deux, il est nécessaire de déterminer la teneur en fer des hépatocytes dans une biopsie percutanée du foie pour établir un diagnostic de surcharge en fer.

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Hemochromatosis should be considered in every patient with cirrhosis of the liver, unexplained pigmentation of the skin, intractable heart disease, diabetes mellitus or impotence. Early detection and treatment of hemochromatosis prevent the development of these complications.

The diagnosis of hemochromatosis requires the demonstration of excessive accumulation of iron in the body. A single elevated serum iron value is of little significance, for 3.5% of ostensibly healthy adults have serum iron values in excess of 200 μg/dL, and primary hemochromatosis can develop without an elevation in serum iron concentration.[1-3] Although an increase in iron saturation of transferrin may indicate excess body iron, such an increase can be due to liver disease and other conditions, and the saturation may be normal despite considerable iron accumulation.[4,5]

Measurement of the serum ferritin concentration has been useful in the diagnosis of clinically manifest hemochromatosis[6-8] and in the detection of hemochromatosis in asymptomatic relatives;[5,9] however, serum ferritin values have been found to be within normal limits in some families with primary hemochromatosis,[10] and the values have been reported to be no more predictive of tissue iron content than transferrin saturation.[11] Moreover, the serum ferritin concentration is elevated in liver disease and in other conditions in the absence of excess body iron.[7,8,12] The ratio of the concentrations of serum ferritin and serum glutamic oxaloacetic transaminase (SGOT) is an empirical index that correlates with the hepatic iron concentration in persons with greatly excessive accumulation of iron in body tissues.[8] There have been no reports of the use of this index in the diagnosis of early iron overload. The percent absorption of a test dose of radioactive iron has not been a useful measure, for it is increased in the early stages of liver disease but may fall to within the normal range in the later stages, when the serum ferritin concentration is markedly increased.[13,14]

Since none of the available tests appears to be entirely satisfactory by itself, the diagnosis continues to rest on histologic or biochemical determination of the amount of iron in the liver.[5]

The study described in this paper was designed to determine the efficacy of the determination of serum iron concentration, transferrin satu-

ration, serum ferritin concentration and serum ferritin:SGOT index in the detection of iron overload in patients with clinical evidence of chronic liver disease — symptoms, signs or abnormal results of liver function tests. Because increased iron absorption is at the root of iron overload, the usefulness of measuring the percent absorption of ingested radioactive iron in relation to the respective serum ferritin concentration was also investigated.

Subjects

Patients with primary hemochromatosis

Studies were done in eight untreated patients with clinically manifest idiopathic, or primary, hemochromatosis (Table I). Three had family members with primary hemochromatosis. Either they presented with clinical features of liver disease, or unexplained abnormalities of liver function were found during the investigation of other complaints. Excess body iron was established by the presence of stainable iron in more than 50% of parenchymal cells in a liver biopsy specimen, and by the subsequent removal of at least 5 g of iron by phlebotomy.

Patients with cirrhosis and iron overload

Investigations were performed in 12 patients with cirrhosis of the liver and stainable iron in more than 50% of parenchymal cells in a liver biopsy specimen (Table I). None had a family history of iron overload. The clinical picture was dominated by signs of liver disease and abnormalities of liver function, and the extent of the liver damage was out of proportion to the degree of hepatic hemosiderosis as judged by light microscopy of a liver biopsy specimen. Chronic alcoholism was noted in six patients.

Patients with liver disease and low to normal hepatic iron stores

Twenty patients with liver disease were selected in whom a specimen from a recent liver biopsy had shown stainable iron in fewer than 50% of parenchymal cells and who were willing to undergo an iron absorption test (Table I). All had a stable hemoglobin concentration and no visible blood in the stool at the time of the study and therefore were not overtly bleeding.

Controls

Studies were done in 34 healthy volunteers to establish a normal range of values for serum ferritin, serum ferritin:SGOT index and iron absorption (Table I).

Informed consent

Informed consent for the investigations was obtained from the patients and the controls.

Methods

Hemoglobin concentration

Hemoglobin concentration was measured with a Coulter Counter. Normal values are 13.5 to 18.0 g/dL for men and 11.5 to 14.0 g/dL for women.

Serum iron concentration and transferrin saturation

Serum iron concentration and iron-binding capacity were measured with a diagnostic kit (Hoffmann-La Roche Ltd., Vaudreuil, PQ). The normal range of values given by the manufacturer for these tests was 65 to 175 μg/dL and 16% to 55% respectively. For this study we considered a serum iron concentration greater than 175 μg/dL and a transferrin saturation greater than 55% as increased.

Serum ferritin concentration

The method described by Luxton and colleagues[15] was used for the analysis of serum. The results did not differ significantly from those obtained previously with the two-site assay.[12,16] For this investigation the upper limit of normal for the serum ferritin concentration was the 90th percentile for Canadians who parti-

Table I—Clinical features of the subjects

Subject group*	Total no.	No. with chronic alcoholism	Age, mean (and range), yr	Hemoglobin, mean (and range), g/dL	SGOT,† mean (and range), mU/mL	Hepatic histopathologic findings	No. with iron overload confirmed by phlebotomy
Controls	34	0	23 (17–38)	12 F: 13.6 (12.3–14.0) 22 M: 15.6 (14.0–16.9)	21 (17–25)	–	–
Patients with:							
Primary hemochromatosis	8	0	50 (21–74)	1 F: 13.6 7 M: 15.1 (11.9–16.5)	59 (41–73)	4 periportal fibrosis 4 cirrhosis	7
Cirrhosis and 3+ to 4+ hepatocellular iron content	12	6	63 (56–71)	2 F: 11.2 (10.9–11.5) 10 M: 12.5 (8.4–14.4)	107 (31–378)	6 alcoholic cirrhosis 4 cryptogenic cirrhosis 1 sideroblastic anemia and cirrhosis 1 porphyria cutanea tarda and cirrhosis	7
Liver disease and 0 to 2+ hepatocellular iron content	20	16	54 (33–74)	8 F: 9.9 (8.7–14.0) 12 M: 13.2 (9.4–16.5)	69 (20.161)	2 fatty liver 16 alcoholic cirrhosis 2 cryptogenic cirrhosis	–

*Grading of iron content of hepatocytes: 0 = no iron; 1+ = iron in occasional cells; 2+ = iron in fewer than 50% of cells; 3+ = iron in more than 50% of cells; 4+ = iron in all cells in massive amounts.
†Serum glutamic oxaloacetic transaminase.

cipated in the Nutrition Canada survey;[16] for men this varied from 90 ng/mL at age 20 years to 350 ng/mL at age 50 years and over, and for women 45 ng/mL at age 20 years to 200 ng/mL at age 70 years and over.

Serum ferritin:SGOT index

The SGOT concentration was determined on an LKB-8600 rate reaction analyser with the use of Plus Chem SGOT reagent (Smith Kline Instruments Inc., Sunnyvale, California). The median and range of normal values were 20 and 10 to 30 mU/mL. For this study an upper limit of normal for the index was derived by dividing the 90th percentile for the serum ferritin concentration by 20; for men the upper limit was 4.5, 12, 15 and 18 at ages 20, 30, 40 and 50 years and over respectively, and for women it was 2.3, 3.5, 4.5, 5.0, 7.5 and 10 at ages 20, 30, 40, 50, 60 and 70 years and over respectively.

Iron absorption

A test dose of 10 μmol of ferrous ascorbate labelled with 1 μCi of iron-59 was given by mouth after the subject had fasted overnight, and the proportion of the dose retained was measured 14 days later with a whole-body counter.[17] For this study 52% was chosen as the upper limit of normal in accordance with the results reported by Heinrich[17] in persons with normal bone marrow stores of iron.

Iron absorption in relation to serum ferritin concentration

When the individual values for iron absorption in the controls were expressed in relation to the respective serum ferritin concentrations a highly significant inverse relation ($r = 0.877$, $P < 0.001$) was found between the percent iron absorption and the logarithmic serum ferritin concentration. This relation permitted the definition of 95% confidence intervals for "appropriate" percentages of iron absorption for given serum ferritin values and for given serum ferritin:SGOT indexes.

Hepatic iron

Liver biopsy sections were stained for hemosiderin with Prussian blue and coded. Iron content of hepatocytes was graded by three of us who had no knowledge of the clinical data, as follows: 0 = no iron; 1+ = iron in occasional cells; 2+ = iron in fewer than 50% of the cells; 3+ = iron in more than 50% of the cells; and 4+ = iron in all the cells in massive amounts. There are difficulties in interpreting hepatocellular iron content; 72% of our sections were assigned the same grade but the other 28% differed by one grade. When there was disagreement the three grades were averaged and the result converted to the nearest whole number. In accordance with the experience of Barry,[18] grades of 0 to 2+ were considered to indicate low to normal hepatic iron stores, and grades of 3+ to 4+ excessive hepatic iron.

Analysis of data

The sensitivity, specificity, efficacy and predictive value of the diagnostic tests were determined,[19] with the grade of hepatocellular iron content as a benchmark of iron overload.

Values are reported as means ± standard deviation. The statistical significance of the difference between any two means was determined by Student's *t*-test.[20] To define the relation between iron absorption and serum ferritin concentration we used the least-squares method of linear regression to find the best-fitting straight line.[20]

Results

Serum iron

The mean serum iron concentration in the patients with excessive hepatic iron, 169 ± 61 μg/dL, was significantly greater ($P < 0.01$) than the mean in the patients with liver disease and low to normal hepatic iron stores, 95 ± 49 μg/dL, but there was considerable overlap of values between the groups (Fig. 1).

Transferrin saturation

All the patients with primary hemochromatosis and 8 of the 12 with iron overload and excessive hepatic iron had transferrin saturation in excess of 55% (Fig. 2). Two patients with liver disease and low to normal hepatic iron stores had elevated transferrin values. The average transferrin saturation was significantly greater ($P < 0.01$) in the patients with iron overload, 74% ± 21%, than in those with low to normal hepatic iron stores, 31% ± 17%.

Serum ferritin

The mean serum ferritin concentration was 2221 ng/mL in the patients with primary hemochromatosis or cirrhosis with excessive hepatic iron, but only 264 ng/mL in patients with liver disease and low to normal hepatic iron stores. In the first two groups all the individual values were greater than 300 mg/mL, but in the third group only 8 of the 20 patients had values greater than 300 ng/mL (Fig. 3).

Serum ferritin:SGOT index

This index did not clearly separate the patients with excessive hepatic iron from those with low to normal

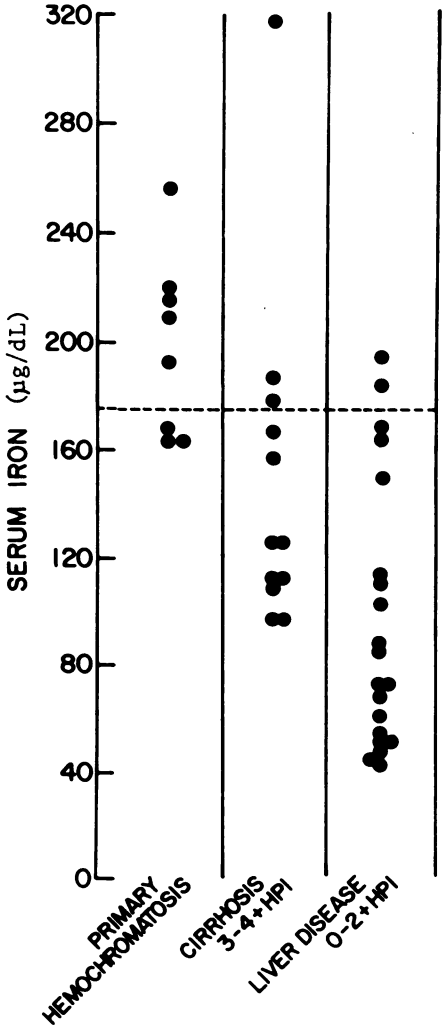

FIG. 1—Serum iron concentration in patients with primary hemochromatosis or liver disease. Broken line indicates upper limit of normal in this study. HPI = hepatic parenchymal iron; 0–2+ = low to normal amounts; 3–4+ = excessive amounts.

stores, and there was considerable overlap of values between the groups (Fig. 4).

Iron absorption

Similarly the values for iron absorption did not differentiate patients with excessive hepatic iron and those with low to normal hepatic iron stores.

Iron absorption in relation to serum ferritin

When the individual values for iron absorption were plotted against the respective serum ferritin values there was a clear distinction between the results for the control subjects and those for the patients with primary hemochromatosis (Fig. 5). A comparable separation of the healthy controls from the patients with primary hemochromatosis was obtained when iron absorption was plotted against the serum ferritin:SGOT index, except that one patient with an iron absorption of 5% had an index of 10 (Fig. 6).

When iron absorption was plotted against the serum ferritin concentration or the serum ferritin:SGOT index (Figs. 5 and 6) the results for the patients with cirrhosis and excessive hepatic iron were all above the upper limit of the 95% confidence intervals for control results.

When iron absorption was plotted against the serum ferritin concentration of the patients with liver disease and low to normal hepatic iron stores the results for 8 of the 20 patients were above the upper limit of the 95% confidence interval for control results (Fig. 5). When iron absorption was plotted against the serum ferritin:SGOT index of these 20 patients two results were above the upper limit of the 95% confidence interval.

Sensitivity and specificity of tests

The frequency of true-positive results (sensitivity) and true-negative results (specificity), based on the findings in the 20 patients with low to normal hepatic iron stores and the 20 patients with excessive hepatic iron, is given in Table II. None of the tests was entirely satisfactory. The combination of determining transferrin saturation and serum ferritin concentration provided high sensitivity with moderate specificity. The iron absorption test by itself lacked specificity and sensitivity; however, with the values expressed in relation to the serum ferritin concentration high sensitivity was achieved, though specificity was lacking because the serum ferritin concentration is elevated out of proportion to the size of the hepatic iron stores in patients with active hepatocellular disease.[8] With iron absorption expressed in relation to the serum ferritin:SGOT index both sensitivity and specificity were high in patients with active hepatocellular disease.

Predictive value of tests

The percentage of all results that were true, whether positive or negative, was calculated for each test or combination of tests on the basis of an assumed prevalence of iron overload of 10% in patients with liver disease. The results (Table III) show that the predictive value of a negative result was 97% to 100% for the determination of transferrin saturation or serum ferritin concentration, or a combination of the latter and other tests. The predictive value of a positive result was less than 50% for any one test.

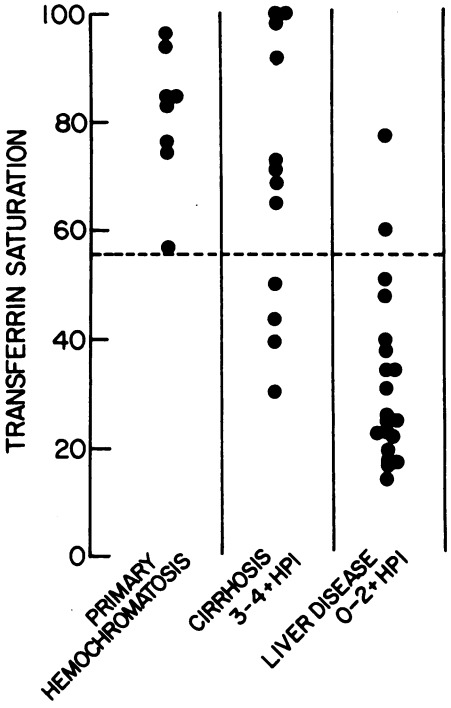

FIG. 2—Transferrin saturation in same groups as in Fig. 1.

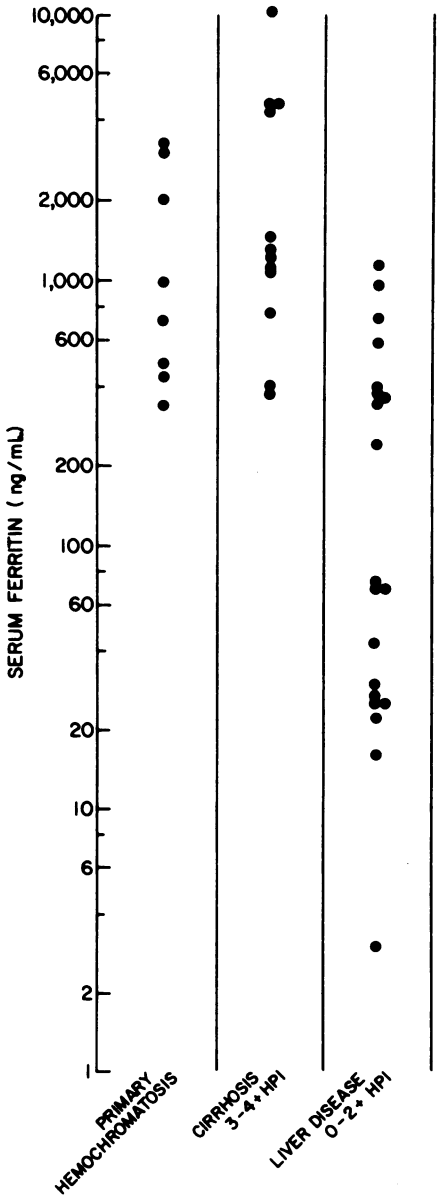

FIG. 3—Serum ferritin concentration in same groups.

Discussion

Determination of the serum iron concentration was of little value in the detection of body iron overload. Transferrin saturation provided a sensitive index of excessive accumulation of iron in body tissues in patients with primary hemochromatosis, but the values were within the normal range in one third of the patients with cirrhosis and iron overload. Furthermore, two patients with liver disease had increased transferrin saturation in the absence of excessive hepatic iron. Determination of transferrin saturation has limited use because of the wide range of normal values and the fact that excessive amounts of iron may accumulate in body tissues without a substantial increase in the serum iron concentra-

tion or a reduction in the transferrin saturation. In addition, conditions such as liver disease reduce transferrin concentrations in the serum and thereby increase transferrin saturation in the absence of iron overload. Despite these limitations the determination of transferrin saturation as a screening test for excessive body iron in our patients was as efficacious as any other single test.

One of the limitations in previous studies[5,9] of the determination of serum ferritin concentrations for the detection of hemochromatosis has been the failure to relate the values to age. When values in our patients were matched for age and sex against those in the general population it became evident that the serum ferritin concentration was a sensitive index of clinically manifest hemochromatosis; all but one patient, with a value of 340 ng/mL, had values greater than the 90th percentile of the general population. In this respect our results resemble those of Powell and Halliday,[21] Edwards and colleagues,[5] and Halliday and asso-

Table II—Diagnostic efficacy of tests for the detection of iron overload, based on the findings in the 20 patients with low to normal hepatic iron stores and the 20 patients with excessive hepatic iron

Measure	Sensitivity (true-positive)*	Specificity (true-negative)†	Efficacy‡
Serum iron concentration	40	90	65
Transferrin saturation (TS)	80	90	85
Serum ferritin (SF) concentration	95	60	78
SF:SGOT index	75	80	78
Iron absorption	45	70	58
TS and SF in parallel	100§	60¶	80
TS and SF:SGOT index in parallel	95§	70¶	83
Iron absorption plotted against SF	100	60	80
Iron absorption plotted against SF:SGOT index	95	90	93

*Percentage of subjects with excessive hepatic iron and an abnormal result.
†Percentage of subjects with low to normal hepatic iron stores and a normal result.
‡Percentage of all subjects with a true-positive or a true-negative result.
§Results of one or other test, or both, positive.
¶Results of both tests negative.

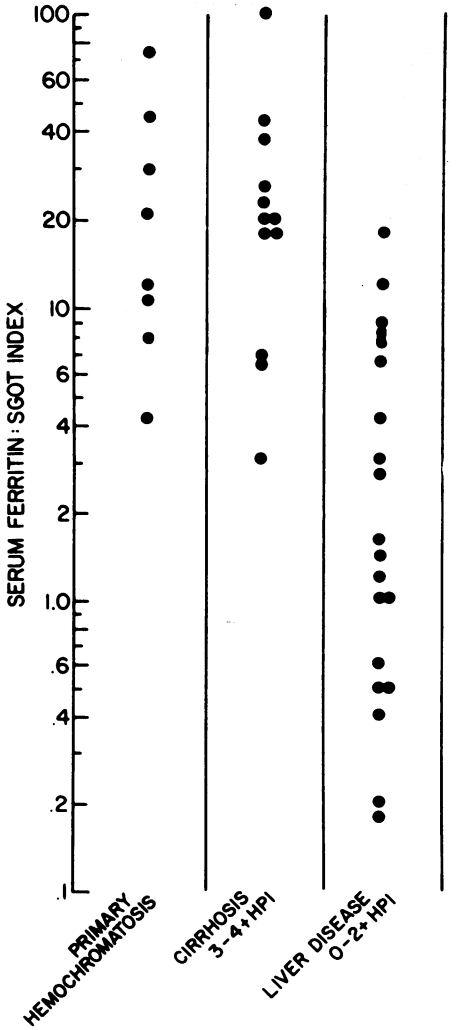

FIG. 4—Serum ferritin:SGOT index in same groups.

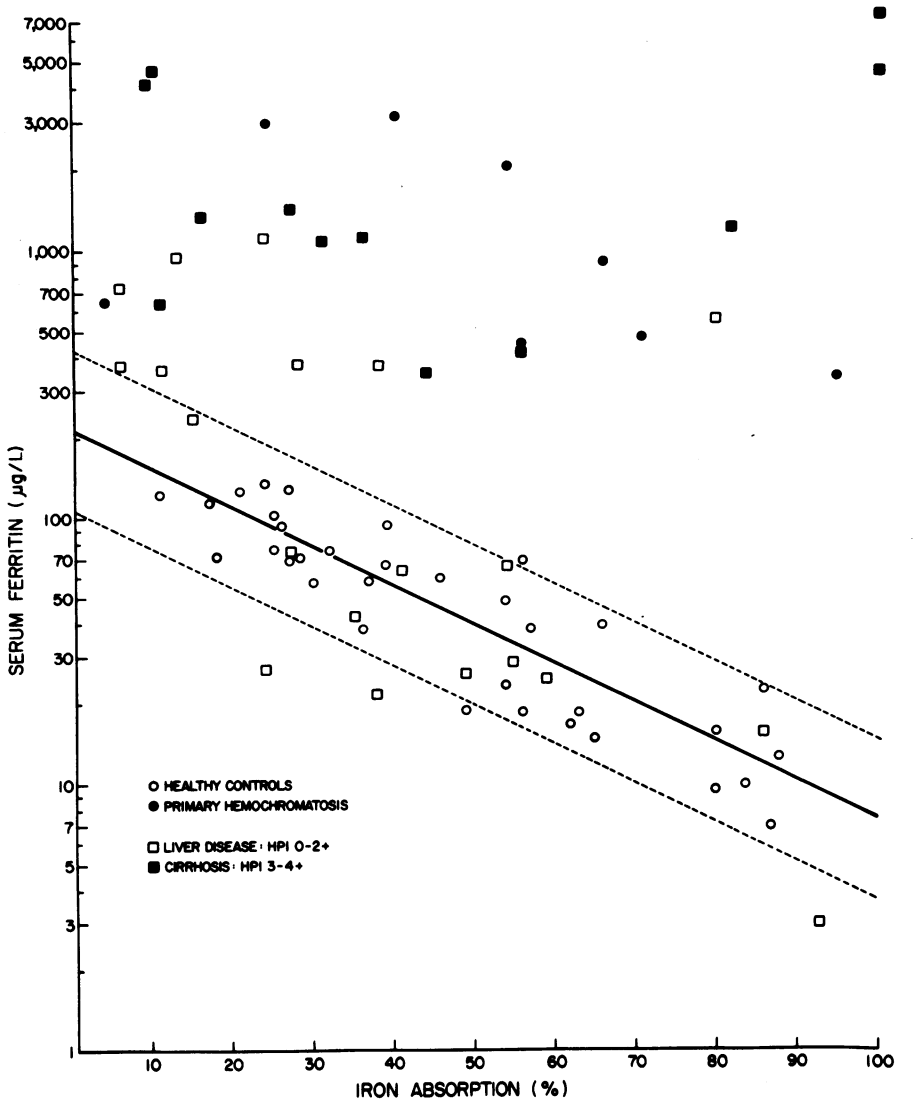

FIG. 5—Relation between iron absorption and serum ferritin concentration in healthy controls, patients with primary hemochromatosis and patients with liver disease. Broken lines indicate 95% confidence limits of control results.

ciates,[9] who found increased serum ferritin values in most of their patients with primary hemochromatosis. Determination of the serum ferritin concentration lacked specificity, however, in patients with active hepatocellular disease, in whom the serum ferritin concentration was increased disproportionately to the size of the body iron stores.[7,9]

Prieto, Barry and Sherlock[8] reported a close correlation between the serum ferritin concentration and an empirical index derived from the product of the serum aspartate transaminase and liver iron concentrations, which implies that the circulating amount of ferritin depends upon both the degree of hepatocellular injury and the size of the liver iron stores. They proposed that the serum ferritin:transaminase ratio may be useful for diagnostic purposes. The use of the serum ferritin:SGOT ratio in our patients increased the specificity of the determination of serum ferritin concentrations in patients with active hepatocellular disease at the expense of sensitivity (Table II).

Since an increase in iron absorption is fundamental to primary hemochromatosis, measurement of iron absorption should be an efficacious test for detection of the disease. This has not proved to be the case in previous studies,[13,14] and in our study

Table III—Estimated predictive value of various tests for the detection of iron overload in comparison with the estimation of hepatocellular iron content*

Measure	Predictive value (%); test result	
	Positive†	Negative‡
Serum iron concentration	31	93
Transferrin saturation (TS)	47	98
Serum ferritin (SF) concentration	24	99
SF:SGOT index	29	97
Iron absorption	12	89
TS and SF in parallel	22	100
TS and SF:SGOT index in parallel	38	99
Iron absorption and SF	22	100
Iron absorption and SF:SGOT index	51	99

*Based on an assumed prevalence of iron overload of 10% in patients with liver disease.
†Percentage of positive results that indicate body iron overload is present.
‡Percentage of negative results that indicate body iron overload is absent.

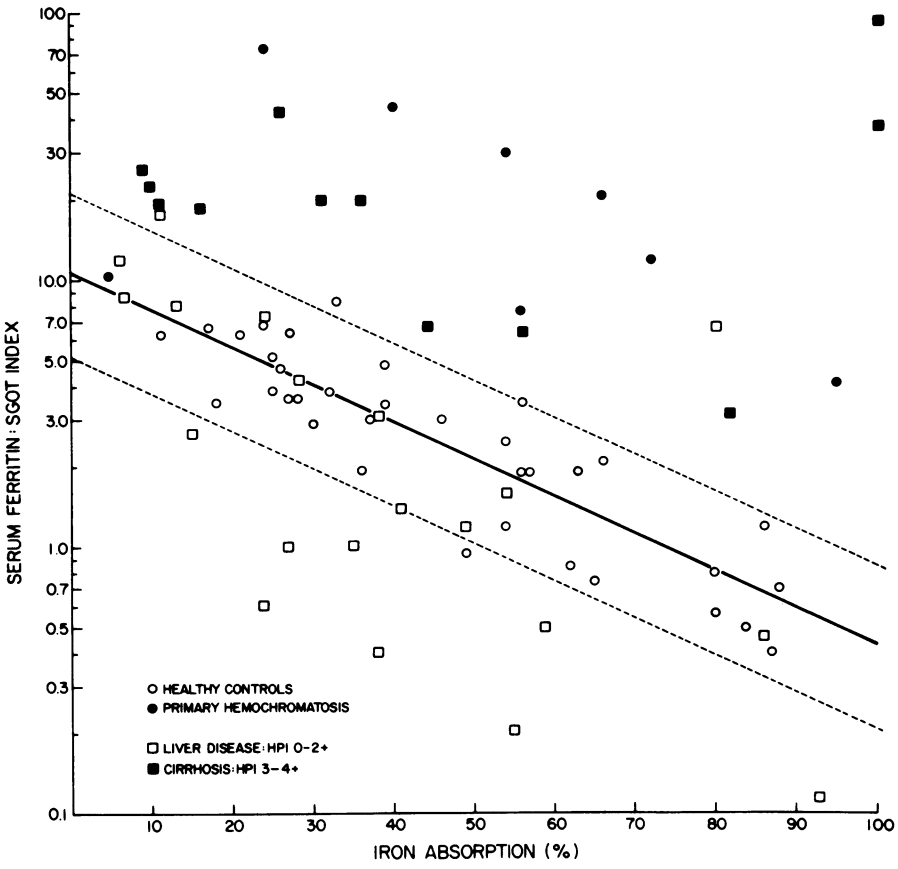

FIG. 6—Relation between iron absorption and serum ferritin:SGOT index in same groups.

this test was neither sensitive nor specific. However, the expression of iron absorption in relation to the size of body iron stores, as measured by the serum ferritin concentration, was a sensitive method for detecting iron overload. This method lacked specificity, though, because of the disproportionate increase in serum ferritin concentration in relation to the size of iron stores in patients with active hepatocellular disease. The use of the serum ferritin:SGOT index as a measure of the size of iron stores partly overcame this problem. Only 2 of the 20 patients with low to normal hepatic iron stores had values above the upper limit of the 95% confidence interval for control values, compared with 8 of the 20 when the serum ferritin concentration was the reference. One of these two patients probably had an iron-loading disorder in the early stages of development; the percent iron absorption was 80%, the serum ferritin value 579 ng/mL and the hepatocellular iron content 2+. He was subsequently found, by repeated venesection, to have body iron stores in excess of 3.5 g. Thus, the expression of iron absorption in relation to the serum ferritin:SGOT index was a method of high sensitivity and moderately high specificity.

The predictive value of tests to detect iron overload is closely related to the prevalence of hemochromatosis in the population scrutinized,[18] as is illustrated in Table III for a selected group with a 10% prevalence of the disease. If a population with a lower prevalence of hemochromatosis — for example, all persons with diabetes — were being screened, the predictive values of the positive test results would be correspondingly lower. Our results indicate that the predictive value of a normal (negative) result for the determination of transferrin saturation, serum ferritin concentration or serum ferritin concentration in combination with other measures is very high for excluding iron overload, but the predictive value of a positive result for these tests is low. Although the combination of tests for iron absorption and the serum ferritin:SGOT index was the most efficacious method, the predictive value of a positive result was not high enough to give this method any practical advantage over the simpler and more convenient tests such as determination of transferrin saturation and serum ferritin concentration for screening patients with chronic liver disease. Further investigation is required to establish the predictive value of expressing iron absorption in relation to the serum ferritin concentration for the early detection of iron overload in asymptomatic relatives of patients with primary hemochromatosis.

In conclusion, histologic appraisal of hepatocellular iron content in a liver biopsy specimen is indicated whenever biopsy is carried out for the histologic diagnosis of liver disease, irrespective of the transferrin saturation and serum ferritin concentration. When a biopsy is not required for this purpose, determination of the transferrin saturation and serum ferritin concentration is useful in screening for iron overload in persons with clinical evidence of liver disease. If the two values are within normal limits it is highly unlikely that iron overload is present. If one or other value is abnormal, then determination of hepatocellular iron content in a specimen from a percutaneous liver biopsy is required to establish the size of the iron stores.

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